Biooxidation of Primary Alcohols to Aldehydes through Hydrogen Transfer Employing Janibacter terrae

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Biooxidation of Primary Alcohols to Aldehydes through Hydrogen Transfer Employing *Janibacter terrae*

Thomas Orbegozo,[a] Johannes G. de Vries,[b] and Wolfgang Kroutil*[a]

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Chemoselective oxidations still represent a challenge for chemists. Lyophilized cells of *Janibacter terrae* were employed for the chemoselective oxidation of primary alcohols to the corresponding aldehydes by hydrogen transfer with the use of acetaldehyde as the hydrogen acceptor. Secondary alcohol moieties were transformed at a much slower rate. The substrate spectrum encompasses substituted benzyl alcohols, whereby substrates with a substituent in the *meta* position were well tolerated, whereas only very small substituents were tolerated in the *ortho* position. Furthermore, *n*-alkanols and allylic alcohols were transformed with good conversions. The biocatalyst was compatible with DMSO as a water miscible organic solvent up to 30 % v/v.

Introduction

The selective oxidation of alcohols to yield carbonyl compounds belongs to the standard repertoire in synthetic organic chemistry. In the search for alternatives driven by the immaturity of many organic oxidation reactions,[1] a lot of emphasis has been put on the development of "green" chemical processes.[2] For instance, laccases have been employed for the oxidation of primary alcohols to aldehydes in combination with mediators.[3] Other biocatalysts like alcohol dehydrogenases, mono-oxygenases, and oxidases have been employed for the chemoselective oxidation of primary alcohols to aldehydes.[4]

We recently reported on the optimization of the chemoselective bio-oxidation of benzyl alcohol to benzaldehyde, employing lyophilized cells of *Janibacter terrae* DSM 13953 as biocatalyst in a hydrogen-transfer process.[5] For this reaction, acetaldehyde was chosen as the most efficient hydride acceptor.

Here we report an extensive study on the substrate spectrum of this biocatalyst including various substituted benzyl alcohols, heteroaromatic carbinols, *n*-alkanols, and allylic alcohols. Furthermore, the compatibility of this biocatalyst with organic solvents was investigated.

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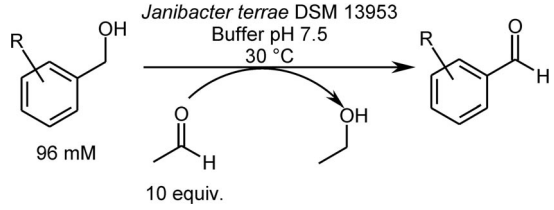
Results and Discussion

To get a clear picture of the positional effect and the type of substituents on the conversion, various *ortho*-, *meta*-, and *para*-substituted benzyl alcohol derivatives were tested in the biocatalytic oxidative hydrogen transfer process, employing lyophilized cells of *Janibacter terrae* as the catalyst and acetaldehyde as the final hydrogen acceptor (Scheme 1).



Scheme 1. Biocatalytic oxidation of substituted benzyl alcohols, employing acetaldehyde as hydrogen acceptor.

The results showed that benzyl alcohols with substituents in the *meta* position were better substrates than those with substituents in the *ortho* or *para* positions (Table 1). *ortho*-Substituted benzyl alcohols were not transformed at all except those with small substituents such as F, Me, and OH.

The size of the substituent in the *para* position had also a significant effect on the conversion: From the results obtained it could be concluded that substrates with smaller substituents are transformed faster than substrates with larger substituents (F > Cl > Br > I; Me > Et). This was supported by results with further *para*-substituted benzyl alcohols: for instance, a phenyl, *tert*-butyl, or *n*-butyl group in the *para* position led to conversions below 5% (not shown in Table 1). In contrast, larger substituents were accepted in the *meta* position, for example, *m*-bromobenzyl alcohol was equally well transformed as the *m*-fluoride ana-

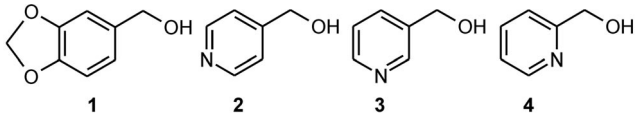
Table 1. Conversion of monosubstituted benzyl alcohols (Scheme 1).[a,b]

R	*para*-R [%]	*meta*-R [%]	*ortho*-R [%]
F	72[c]	73	23[c]
Cl	11[d]	51	<1
Br	6	73	<1
I	2	88[c]	<1
Me	21[d]	96[c]	9[d]
Et	6	n.d.[e]	<1
OMe	46[d]	84	<1
OH	5	22	22
NO_2	8[d]	22	<1

[a] For comparison: R = H, benzyl alcohol was oxidized with 95% conversion. Conversions were measured by GC–MS. [b] Reaction conditions: Pi buffer (100 mM, pH 7.5), 96 mM alcohol, 10 equiv. acetaldehyde, 30 °C, 24 h, 3 mL total volume, 100 mg cells. [c] Data reported in ref.[5] [d] Solid substrates were dissolved in acetaldehyde prior to addition. [e] n.d. not determined, because the substrate is not commercially available.

logue. The *m*-iodide derivative led to even higher conversion than the *m*-bromide compound. Benzyl alcohols carrying *m*-methyl or *m*-methoxy substituents were also well transformed. No clear electronic effect could be deduced from these results. The high tolerance for substituents in the *meta* position is supported by the results obtained with substrates like *m*-CF_3- and *m*-NMe_2-benzyl alcohol (61 and 40% conversion, respectively; not shown in Table 1).

Conversions of disubstituted benzyl alcohols generally remained below 3% (2,4-dimethyl, 3,5-dimethyl, 2-chloro-5-nitro). One remarkable exception is piperonyl alcohol (**1**), which was oxidized to the corresponding aldehyde with 51% conversion. 3,4-Dimethylbenzyl alcohol was oxidized with 8% conversion.



To test if heteroaromatic analogues can also be converted, the regioisomers of pyridinemethanol were investigated. The highest conversion was achieved with the isomer where the nitrogen is in the *meta* position (substrate **3**, 61%), whereas the other isomers led to low conversions (**2**: 13%; **4**: 16%).

Switching to aliphatic *n*-alkanols, we found that substrates containing up to eight carbon atoms were transformed rather well (Figure 1, up to 81% conv.). Longer chains led to conversions around 45% (Figure 1).

In comparison with *n*-pentanol, the related branched alcohol 3-methyl-1-butanol (**5**) was transformed somewhat slower (62% instead of 72% conv. for 1-butanol; Table 2). A second methyl group as in substrate **6** led to even lower conversion (54%). Hydrogenated benzyl alcohol, such as cyclohexylmethanol (**7**) and the corresponding five-membered carbocycle cyclopentylmethanol (**8**) were transformed equally well (63 and 68%).

ω-Phenyl-1-alkanols like substrates **9** and **10** led only to moderate conversions (40 and 18%, respectively). However, allylic alcohols were in general very good substrates; for

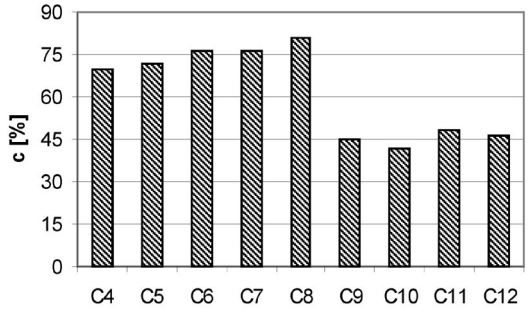


Figure 1. Biocatalytic oxidation of *n*-alkanols to the corresponding aldehydes through hydrogen transfer (10 mL L^{-1} alcohol, 10 equiv. acetaldehyde, 30 °C, 24 h).

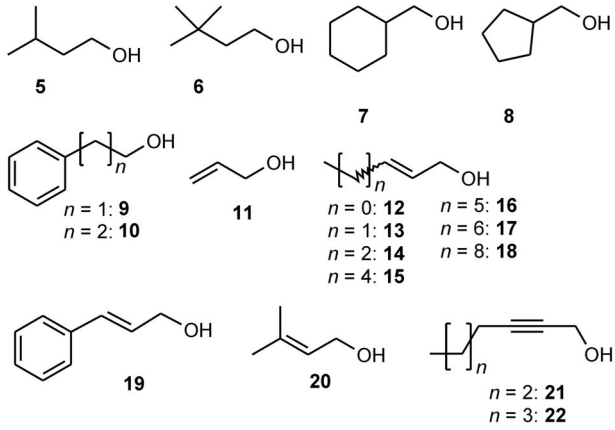


Table 2. Bio-oxidation of aliphatic, allylic, and acetylenic alcohols.[a]

Substrate	Conv. [%]	Substrate	Conv. [%]	Substrate	Conv. [%]
5	62	**12**[b]	>99	*trans*-**17**	69
6	54	*cis*-**13**	98	*trans*-**18**	40
7[c]	63	*trans*-**13**	95	*trans*-**19**	36
8[c]	68	*cis*-**14**	99	**20**	>99
9[c]	40	*trans*-**14**	98	**21**	6
10[c]	18	*trans*-**15**	98	**22**	14
11	>99	*trans*-**16**	86	–	

[a] Reaction conditions: Pi buffer (100 mM, pH 7.5), 10 mL L^{-1} alcohol, 10 equiv. acetaldehyde, 30 °C, 24 h, 3 mL total volume, 100 mg cells; Conversions were measured by GC–MS. [b] *cis*/*trans* mixture. [c] 96 mM.

instance, allylic alcohols **11**–**15** and **20** were transformed with over 90% conversion. Substrates with more than eight carbon atoms in the main chain were oxidized slower, as already observed for *n*-alkanols (Figure 1); this is also true for allylic alcohols **16**–**18**.

Comparing allylic alcohol **15** with corresponding acetylenic alcohol **22**, we observed that the latter led only to 14% conversion, whereas **15** was oxidized with 98% conversion. Thus, the presence of the C≡C bond led to a significantly reduced conversion.

Comparing the oxidation rate of primary alcohols with the oxidation rate of secondary alcohols, a significant preference for primary alcohols was observed. For instance, the

oxidation of 1-octanol occurred 62-times faster than the oxidation of (*R*)-2-octanol and 26-times faster than the oxidation of (*S*)-2-octanol (Figure 2). A related trend was observed for heptanol regioisomers. Interestingly, the oxidation rate increased slightly for more sterically hindered alcohols like *rac*-4-octanol, probably due to some other enzymes present in the biocatalyst preparation.

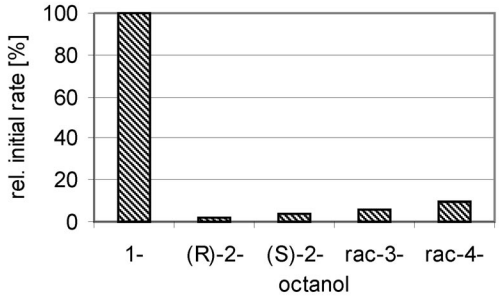


Figure 2. Comparison of rate for the oxidation of octanol regioisomers (64 mM substrate, 15 equiv. acetaldehyde, 30 °C, 2 h).

It is worth to mention that in all cases over-oxidation of the aldehyde to the carboxylic acid was negligible; thus, the biocatalytic transformation is highly chemoselective and only catalyzes the oxidation of the primary alcohol to the corresponding aldehyde under the reaction conditions employed. This is especially noteworthy, as alcohol dehydrogenases have been reported to oxidize aldehyde hydrates.[6] Therefore special attention was put on the detection of carboxylic acids in the hydrogen transfer oxidation of all substrates, but acid formation was in general below detection limits if samples were analyzed quickly after extraction. For commercial acids like benzoic acid, heptanoic acid, phenylacetic acid, and *trans*-2-hexenoic acid it was ensured that if these acids would be presented they would indeed be detected after acidifying the reaction medium and extraction.

Finally, the tolerance of the biocatalyst toward water-miscible organic solvents was evaluated (Figure 3). Water-miscible organic solvents are frequently added to biocatalytic reactions in aqueous solution to improve the solubility

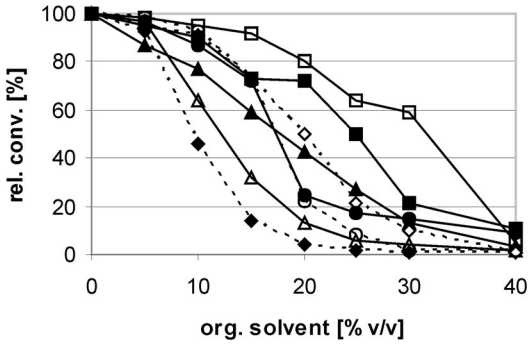


Figure 3. Cosolvent effect in the oxidation of benzyl alcohol in buffer/organic solvent mixtures (96 mM substrate, 7.5 h). Solvents: ♦ THF; □ DMSO; ▲ DMF; Δ acetonitrile; ◇ dioxane; ● 2-propanol; ■ 1-methyl-2-pyrrolidone; ○ acetone. 100% corresponds to 87% conversion.

of lipophilic substrates in the buffer.[7] From the eight solvents studied, DMSO proved to be the most compatible one, whereas THF was the least tolerated. 1-Methyl-2-pyrrolidinone led to comparable good conversions as in DMSO. DMSO can be used up to 30% v/v maintaining 60% of the original conversion. At 40% v/v all solvents led to an almost complete loss of activity. At 10% v/v all solvents tested were tolerated.

Conclusions

Primary alcohols were chemoselectively oxidized to the corresponding aldehydes, employing lyophilized cells of *Janibacter terrae* as catalyst and acetaldehyde as hydrogen acceptor through hydrogen transfer resembling an biocatalytic Oppenauer oxidation[8] or metal-catalyzed hydrogen transfer.[9] The substrate spectrum encompasses substituted benzyl alcohols, whereby the *meta* position is highly preferred, as well as *n*-alkanols and allylic alcohols. The catalyst preferentially oxidizes primary alcohols over secondary alcohol moieties and thus possesses high chemoselectivity. The biocatalyst showed high tolerance toward DMSO as a water-miscible organic solvent up to 30% v/v.

Experimental Section

General: Acetaldehyde, all alcohols, benzoic acid, heptanoic acid, phenylacetic acid, *trans*-2-hexenoic acid, and most aldehydes were purchased from Sigma–Aldrich (Sigma–Aldrich–Fluka, Vienna, Austria). Lyophilized cells of *Janibacter terrae* DSM 13953 were prepared as described previously.[5]

General Procedure for the Bio-Oxidation of Alcohols by Hydrogen Transfer: Lyophilized cells of *Janibacter terrae* DSM 13953 (100 mg) were rehydrated in phosphate buffer (3 mL, 100 mM, pH 7.5) in a glass tube (7.5 mL total volume). The closed glass tube was positioned horizontally on a shaker and agitated at 30 °C, 120 rpm for 30 min. Afterwards, acetaldehyde (160 μL, 0.13 g, 2.85 mmol) and alcohol (appropriate amount given throughout this paper) were added. For solid substrates the alcohol was predissolved in the required amount of acetaldehyde. The reaction was shaken at 30 °C and 120 rpm for 24 h. The reaction was stopped by adding ethyl acetate (3 mL), shaking, and centrifugation (12000 rpm, 2 min). For testing for over-oxidation of the aldehydes, the samples were acidified prior to extraction (10 N HCl). The separated organic phase was dried (Na_2SO_4) prior to determination of conversion by GC–MS.

Bio-Oxidation in the Presence of Water-Miscible Organic Solvents: The experiments were performed as described above, but adapting the amount of buffer and organic solvent in that way, that the total volume corresponds to 3 mL.

Analytics: Conversions were determined by using an Agilent 7890A gas chromatograph equipped with an Agilent 5975C mass-selective detector (electron impact, 70 eV) and an Agilent HP-5ms column [30 m × 250 μm × 0.25 μm, 5%-phenylmethylpolysiloxane phase]. Helium was used as carrier gas at a flow of 2 mL min^{-1}. Products were identified by co-injection with commercial available reference compounds and/or by MS analysis.

Supporting Information (see footnote on the first page of this article): Retention times and mass spectra.

Acknowledgments

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